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Mal-deficiency impairs the tolerogenicity of dendritic cell of patients with allergic rhinitis

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ABSTRACT

The tolerogenic dendritic cell dysfunction is associated with the pathogenesis of immune diseases. Microbial stimulus is required in the maintenance of immune functions. This study aims to elucidate the role of Mal signal in the maintenance of DEC205$^+$ DC (decDC) immune tolerogenic function. In this study, peripheral DCs were collected from allergic rhinitis (AR) patients and healthy control (HC) subjects to assess the functional status of decDCs. An AR murine model was developed to test the role of Mal signals in the maintenance of decDCs' functions. We observed that AR decDCs (decDCs obtained from AR patients) were incompetent in the induction of type 1 regulatory T cells (Tr1 cells). AR decDCs expressed less IL-10 than that in HC decDCs. IL-10 mRNA decayed spontaneously in AR decDCs. Tat-activating regulatory DNA-binding protein-43 (TDP43) protected IL-10 mRNA from decay. AR decDCs expressed lower levels of Mal than that in HC decDCs. Mal depletion resulted in IL-10 mRNA decay in HC decDCs. Reconstitution of Mal in AR decDCs restored the capacity of inducing Tr1 cells and attenuated experimental AR in mice. In conclusion, Mal plays a critical role in the maintenance of decDC's immune tolerogenic function. The absence or insufficient Mal signal impairs decDC's tolerogenic property. Reconstitution of Mal in AR decDCs can restore the immune tolerogenic capacity, which may have translational potential in the treatment of AR and other allergic diseases.

1. Introduction

Allergic rhinitis (AR) is an adverse response to airborne allergens by the immune system in the nasal mucosa. It is estimated that the prevalence of AR is more than 10% in the world [1]. The clinical symptoms of AR attack include nasal itch, sneezing, profound nasal discharge and nasal congestion [2]. Rhinosinusitis is one of the common complications of AR [3]. Allergic asthma may develop following AR [4]. The therapeutic efficacy of AR is unsatisfactory currently [5]. In fact, AR has been a great negative impact on human health and social economy. Thus, it is necessary to further understand the pathogenesis of AR and to invent novel and effective remedies for the treatment of AR.

AR is featured by the T helper (Th)2 polarization in the nasal mucosa [6]. Th2 cells gather in the local tissues and produce large amounts of Th2 cytokines, mainly including IL-4, IL-5 and IL-13. Th2 cytokines, such as IL-4, induce the production of immunoglobulin (IgE) by plasma cells. IgE binds the high affinity IgE receptors on the surface of mast cells to make mast cells sensitized. Re-exposure to specific antigens activates the sensitized mast cells. The mast cells release chemical mediators to evoke AR clinical symptoms [7]. Yet, factors in the initiation of AR are not fully elucidated.

In general, immune responses are tightly regulated by the immune regulatory system in the body. The immune regulatory system includes immune regulatory cells, such as tolerogenic dendritic cells (DCs), regulatory T cells (Tregs) and regulatory B cells (Bregs), and immune regulatory mediators, such as interleukin (IL)-10 and transforming

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Table 1
Demographic data of AR patients.

Item	AR	Healthy
Male/female	10/10	10/10
Age	34.4 ± 3.5	33.5 ± 4.6
Weight	56.8 ± 10.2	56.6 ± 12.6
Height	155.3 ± 11.2	157.8 ± 11.1
AR	20 (100%)	0
AR/asthma	2 (10%)	0
AR/eczema	2 (10%)	0
Total IgE (IU/ml)	428.3 ± 135.2	0.33 ± 0.19
Specific IgE (IU/ml)	68.6 ± 18.2	nd
Current smoker	2 (10%)	2 (10%)

AR: Patients with AR. specific IgE, specific IgE for dust mite. The values are presented as mean ± SD. nd: Not detectable.

growth factor (TGF)-β [8–10]. It is recognized that DEC205$^+$ DCs (decDCs, in short) have an immune tolerogenic function by inducing immune regulatory T cells [11]. Upon activation, Tregs or/and Bregs release immune regulatory mediators to suppress other immune cell activities to avoid inducing tissue injury [12]. It is reported that reduction of Treg or Breg number or dysfunctional immune regulatory system is associated with many immune disorders [13]. The Th2 polarization status and over production of IgE in AR patients mirror the dysfunctional status of the immune regulatory system. Although research about immune regulation advanced rapidly in the last few decades, the maintenance of the homeostasis in the immune regulatory system remains to be further investigated.

After transcription, it is critical to check the fidelity of the mRNA. Those RNAs not processed properly are eliminated. The related protein synthesis is then terminated. This phenomenon is designated RNA decay [14]. To ensure mRNAs to work properly, a fraction of the protein can bind the mRNA to protect them from degrading [15]. These proteins are called RNA-binding proteins. If RNA-binding proteins are not properly produced, the destiny of target RNAs may be affected [15]. Yet, whether improper RNA decay associates with immune regulatory cell dysfunction is to be investigated.

Microbial stimulus is required in the maintenance of immune functions in the body [16]. Absence of microbes in the intestine, e.g., the germ-free mice, can not develop a functional immune system [17]. Regulatory immune cells are reduced in germfree mice [18]. Lipopolysaccharide (LPS) is one of the most common microbial products and has extensive effects on regulating immune activities by activating a specific signal transduction pathway through ligating the Toll-like receptor (TLR)4. MyD88 (myeloid differentiation primary response gene 88) and Mal (MyD88 adaptor-like) are the common check points in TLR4 signal transduction pathway. Mal serves as a “bridge adaptor” for MyD88 to form the Myddosome by recruiting cytosolic MyD88 to interact with the activated TIR (Toll/IL-1R/resistance) domains of TLR4 dimers at the cell membrane; Mal also has a catalyzing role in the formation of the so-called “Myddosome” [19]. The LPS/TLR4 axis plays a critical role in the maintenance of the immune regulatory system [20], in which the role of MyD88 or/and Mal is to be further investigated.

In this study, we intend to elucidate the factors inducing RNA decay of immune regulatory mediators. The results reveal that TLR signals are required in the maintenance of the homeostasis of the mRNA of immune regulatory cells.

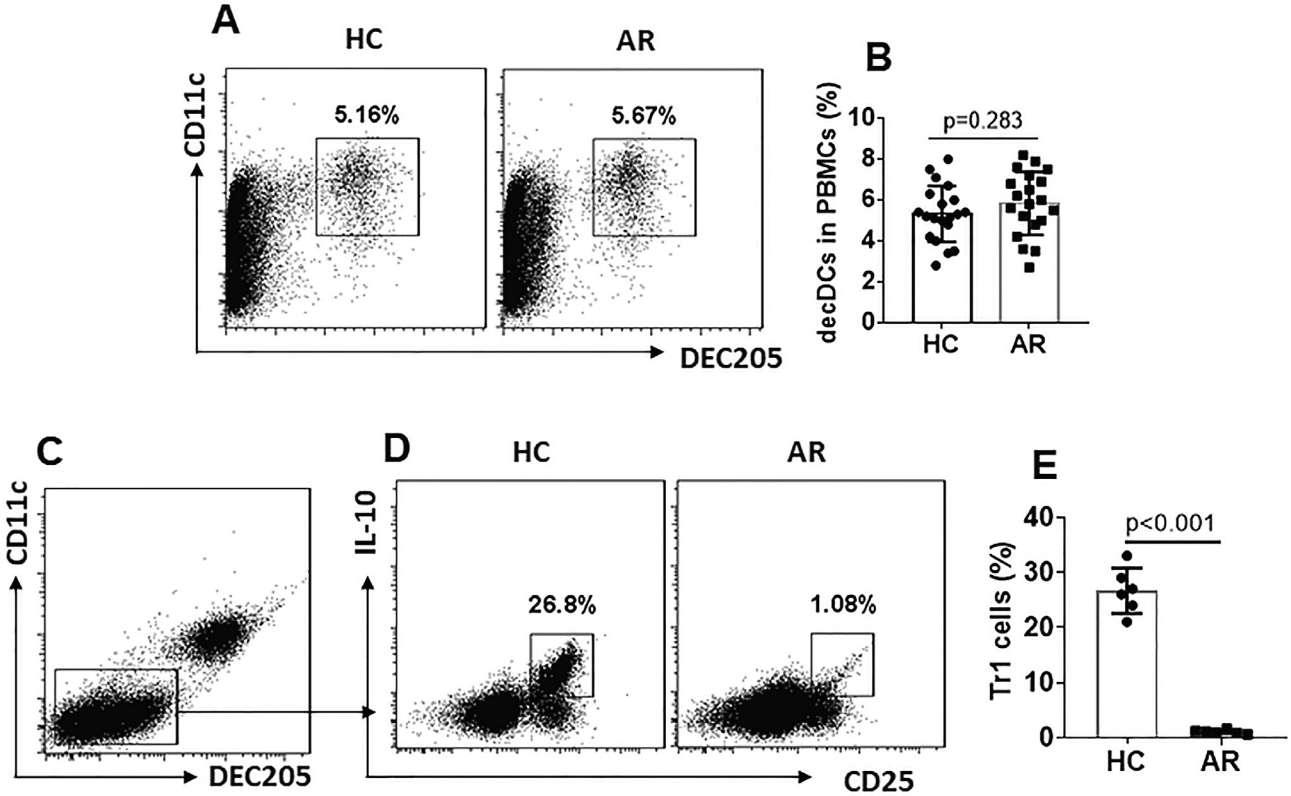

Fig. 1. Assessment of decDC in AR patients and HC subjects. A-B, PBMCs were isolated from blood samples collected from AR patients (n = 20) and HC subjects (n = 20) and analyzed by flow cytometry. A, the gated dot plots show decDCs in PBMCs (the gating strategy is only both CD11c$^+$ and CD205$^+$ cells are gated). B, summarized decDCs in PBMCs of panel A. C-E, decDCs and naïve CD4$^+$ T cells were isolated from PBMCs and cultured at a ratio of 1:5 (DC:T cell) in the presence of IL-2, PMA and ionomycin for 6 days. The cells were analyzed by flow cytometry. C, the gated cells are CD4$^+$ T cells (DCs were gated out). D, the gated dot plots show induced Tr1 cells. E, bars indicate summarized frequency of Tr1 cells of panel D. Data of bars are presented as mean ± SEM. Each dot inside bars presents data obtained from an independent experiment.

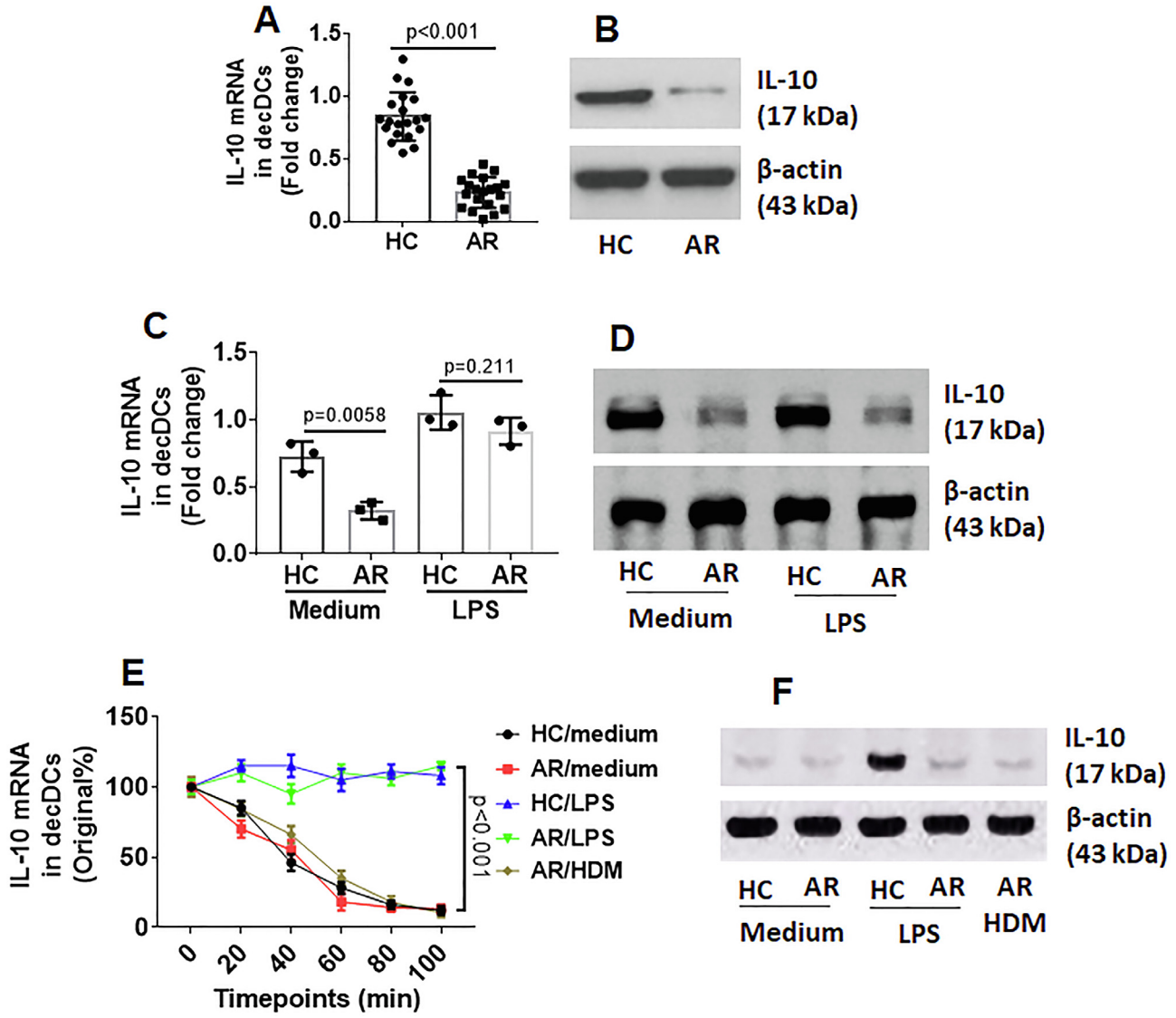

Fig. 2. Expression of IL-10 is impaired in AR decDCs. A-B, decDCs were isolated from PBMCs collected from blood samples of AR patients (n = 20) and HC subjects (n = 20). The decDCs were analyzed by RT-qPCR and Western blotting. The bars indicate IL-10 mRNA and the immunoblots indicate IL-10 protein in decDCs. C-F, decDCs were cultured in the presence of LPS (100 ng/ml) or house dust mite (HDM, 10 μg/ml, generated in our laboratory) for 48 h. C, the bars indicate IL-10 mRNA. D, the immunoblots indicate IL-10 protein in decDCs. E, the curves show IL-10 mRNA decay in decDCs. F, the immunoblots show IL-10 protein levels in decDCs after culturing for 48 h. Data of bars are presented as mean ± SEM. Each dot inside bars presents data obtained from an independent experiment. In panel B, the twenty protein extracts of decDCs of the AR group or the HC group were pooled, respectively, and analyzed by Western blotting. The immunoblots are from one experiment representing 3 independent experiments.

2. Materials and methods

2.1. Human subjects

Patients with perennial AR at the remission stage were recruited at the Affiliated *ENT* Hospital of Shenzhen University (Shenzhen, China). The diagnosis and management of AR were carried out by our physicians following our established procedures. A group of healthy control (HC) subjects was also recruited into this study. The demographic data of human subjects are presented in Table 1. AR patients with any of the following conditions were excluded from this study: Cancer; severe organ diseases; autoimmune diseases; under treatment with immune suppressive agents for any reasons. The using human tissue in the present study was approved by the Human Ethics Committee at Shenzhen University. An informed written consent was obtained from each human subject.

2.2. Reconstitution of Mal in AR decDCs

Mal-expressing plasmids were provided by Shanghai Sangon Biotec (Shanghai, China). AR decDCs were isolated from PBMCs by MACS and transfected with Mal-expressing plasmids or control plasmids following the manufacturer's instructions to reconstitute the expression of Mal. The effects were assessed by Western blotting 48 h after the transfection.

2.3. Detection of TDP43/IL-10 mRNA complex in decDCs

HC decDCs and AR decDCs were prepared and radiated by 0.15 J/cm^2 of 365 nm UV light in a Stratalinker 2400 (Stratagene) to cross-link the RNA/protein in the cytoplasm. The cells were lysed with a lysis buffer following by sonication. The lysates were treated with the IP procedures. After eluting, RNA was recovered from the samples with an RNA extracting reagent kit following the manufacturer's instructions

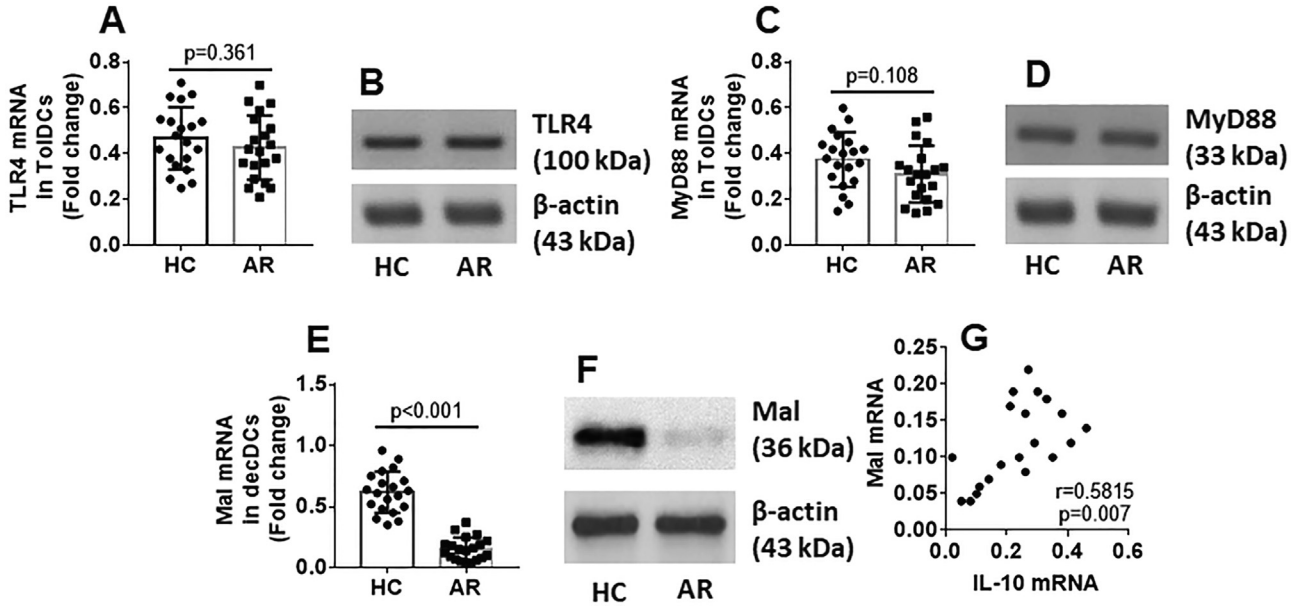

Fig. 3. Levels of TLR4, MyD88 and Mal in decDCs. decDCs were isolated from PBMCs collected from blood samples of AR patients (n = 20) and HC subjects (n = 20). The RNA and protein extracts of decDCs were prepared and analyzed by RT-qPCR and Western blotting. A-B, expression of TLR4. C-D, expression of MyD88. E-F, expression of Mal. G, scatter dot plots show positive correlation between Mal mRNA and IL-10 mRNA (presented in Fig. 2A). Data of bars are presented as mean ± SEM. Each dot inside bars presents data obtained from an independent experiment. The twenty protein extracts of decDCs of the AR group or the HC group were pooled, respectively, and analyzed by Western blotting. The immunoblots are from one experiment representing 3 independent experiments.

and analyzed by RT-qPCR in the presence of IL-10 primers; the results are presented as fold of input. The proteins were analyzed by Western blotting. The procedures were carried out at 4 °C.

2.4. Statistical analysis

The difference between data obtained from two groups was determined by Student *t* test. For multiple comparisons, ANOVA followed by Dunnett's *t* test or Student-Newman-Keuls test was performed. If necessary, the Pearson correlation assay was carried out.

Some experimental procedures are presented in the Supplemental Materials.

3. Results

3.1. Immune tolerogenic functions of AR decDCs are impaired

To understand the functional status of AR decDCs, blood samples were collected from AR patients and HC subjects. Peripheral blood mononuclear cells (PBMCs) were isolated from the blood samples and analyzed by flow cytometry. The frequency of AR decDC (decDCs in the AR group) was comparable with HC decDCs (Fig. 1A-B). To test the tolerogenicity of the decDCs, AR decDCs and HC decDCs were isolated from PBMCs and cocultured with naïve $CD4^+$ T cells. The results showed that Tr1 cells were induced by decDCs in the culture which were much less in the AR group than that of the HC group (Fig. 1C-E). The results demonstrate that the immune tolerogenic functions are impaired in AR decDCs.

3.2. IL-10 synthesis is impaired in AR decDCs

Since IL-10 is the canonical cytokine by which DCs induce Tr1 cells [21], the data of Fig. 1 imply that the expression of IL-10 may be impaired in AR decDCs. To test this, decDCs were isolated from PBMCs and analyzed by RT-qPCR and Western blotting without culture. The results showed that the expression of IL-10 was markedly lower in AR decDCs than that in HC decDCs at both mRNA and protein levels (Fig. 2A-B). The results indicate that the IL-10 expression is impaired in AR decDCs. To verify the results, decDCs were prepared and stimulated with LPS in the culture. The results showed that exposure to LPS increased IL-10 mRNA in both HC decDCs and AR decDCs. But the IL-10 protein was only increased in HC decDCs, not in AR decDCs (Fig. 2C-D). The results suggest that the gene transcription of IL-10 is still functional in AR decDCs while the IL-10 protein synthesis in decDCs is impaired. On the other hand, we also found that only few (1–2%) $DEC205^-$ $CD11c^+$ DCs are $IL\text{-}10^+$ in both HC group and AR group (Fig. S1 in Supplemental Materials).

3.3. IL-10 mRNA decays spontaneously in decDCs

RNA decay is a common phenomenon in the cell [14]. To elucidate the role of RNA decay in the impairment of IL-10 protein synthesis in decDCs, HC decDCs and AR decDCs were prepared and cultured in RPMI1640 medium. IL-10 mRNA was increased in both HC and AR decDCs in the presence of LPS, while after washing and re-culture in fresh medium, the levels of IL-10 mRNA declined gradually in both HC and AR decDCs in a time-dependent manner (Fig. 2E). Since exposure to LPS can induce IL-10 expression in DCs [22], in separate experiments, LPS was added to the culture. The presence of LPS stabilized IL-10 mRNA and protein levels in HC decDCs, but in AR decDCs, it only stabilized mRNA, not protein levels of IL-10 (Fig. 2F). The results demonstrate that IL-10 mRNA decays spontaneously in both HC decDCs and AR decDCs, which can be stabilized by the presence of LPS. However, the synthesis of IL-10 protein in AR decDCs is impaired even though the presence of the IL-10 inducer. Exposure to the specific antigen HDM (house dust mite, decDCs were collected to AR patients sensitized to HDM) did not prevent the IL-10 mRNA decay in decDCs (Fig. 2E-F).

3.4. Lower expression of Mal associates with IL-10 mRNA levels in AR decDCs

The data of Fig. 2 imply defects in the signal transduction pathway of TLR4 in AR decDCs. We then assessed the levels of canonical

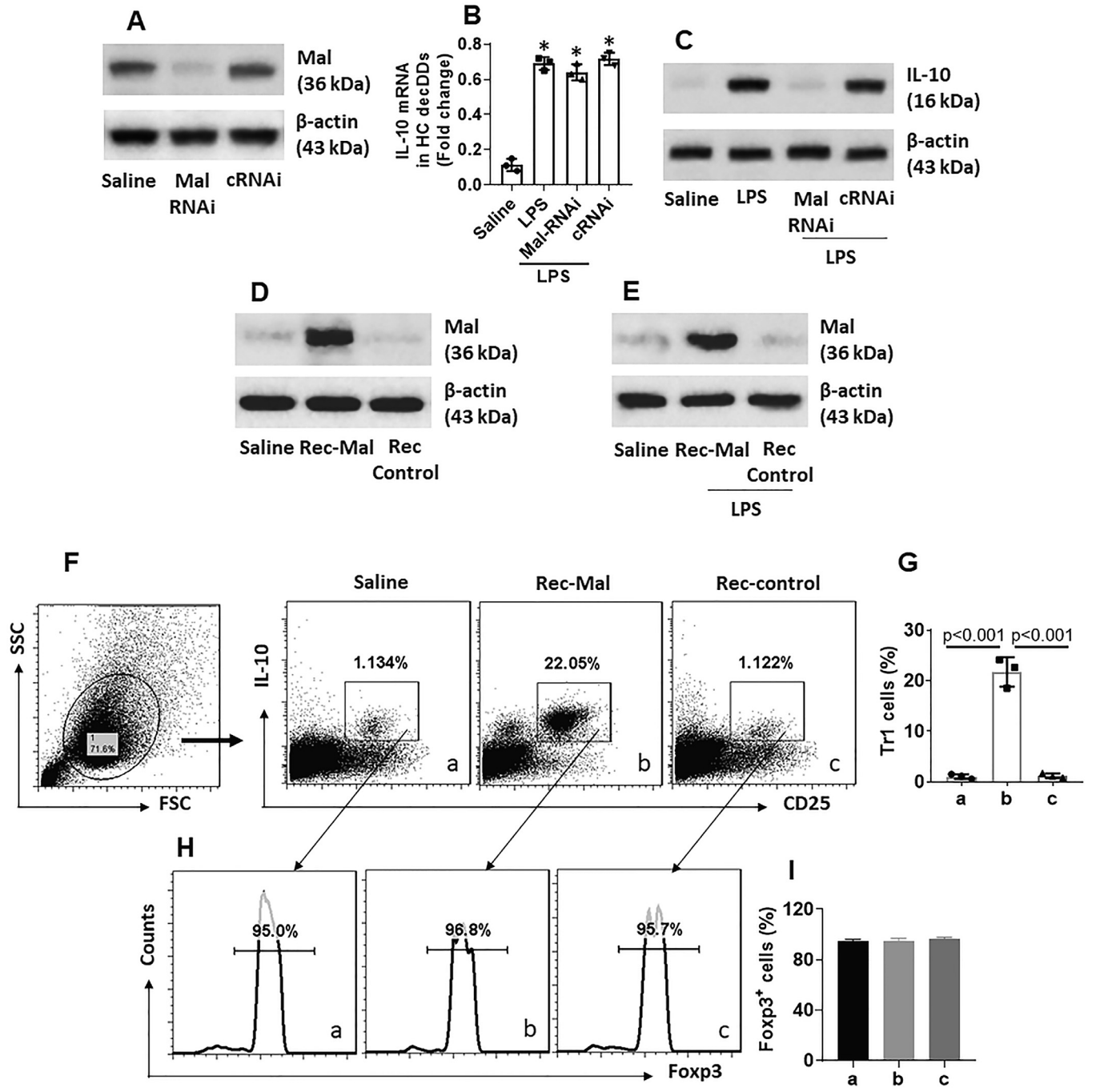

Fig. 4. Modulation of Mal regulates IL-10 expression in decDCs. A, results of Mal RNAi in HC decDCs. B-C, HC decDCs were treated with the procedures denoted on the x axis of panel B. Bars indicate IL-10 mRNA levels (B) and immunoblots indicate IL-10 protein levels (C). LPS: 100 ng/ml. D, reconstitution of Mal (Rec-Mal) in AR decDCs (by transfecting with Mal-expressing plasmids). Rec-control: AR decDCs were treated with empty plasmids used as a control. E, IL-10 protein in AR decDCs after exposure to LPS in the culture. F, AR decDCs and naïve $CD4^+$ T cells were isolated from PBMCs and cultured at a ratio of 1:5 (DC:T cell) in the presence of IL-2, PMA and ionomycin for 6 days. The cells were analyzed by flow cytometry. The gated dot plots show induced Tr1 cells in the total cells (decDCs and $CD4^+$ T cells). G, the bars indicate summarized frequency of Tr1 cells in panel F. H-I, histograms show that the $IL\text{-}10^+$ $CD25^+$ cells are $Foxp3^+$. Bars show summarized data of panel H. Data of bars are presented as mean ± SEM; *$p < 0.001$, compared with the saline group (B). Each dot inside bars present data obtained from one independent experiment. The data of immunoblots and flow cytometry represent 3 independent experiments.

components of the TLR4 signal pathway, including TLR4, MyD88 and Mal, in AR decDCs and HC decDCs. The results showed that the levels of TLR4 (Fig. 3A-B) and MyD88 (Fig. 3C-D) in AR decDCs were comparable with that in HC decDCs, while the levels of Mal in AR decDCs were significantly lower than that in HC decDCs (Fig. 3E-F). A positive correlation was identified between the data of Mal and IL-10 mRNA in AR decDCs (Fig. 3G). The data suggest that the lower expression of Mal may associate with the IL-10 mRNA decay in decDCs.

3.5. Modulation of Mal expression regulates IL-10 mRNA stability in decDCs

To understand the role of Mal in the regulation of IL-10 mRNA stability in decDCs, HC decDCs were prepared and treated with Mal RNAi to knock down the Mal expression (Fig. 4A). The Mal-deficient HC decDCs were exposed to LPS in the culture for 48 h. The cells were then analyzed by RT-qPCR and Western blotting. The results showed that IL-

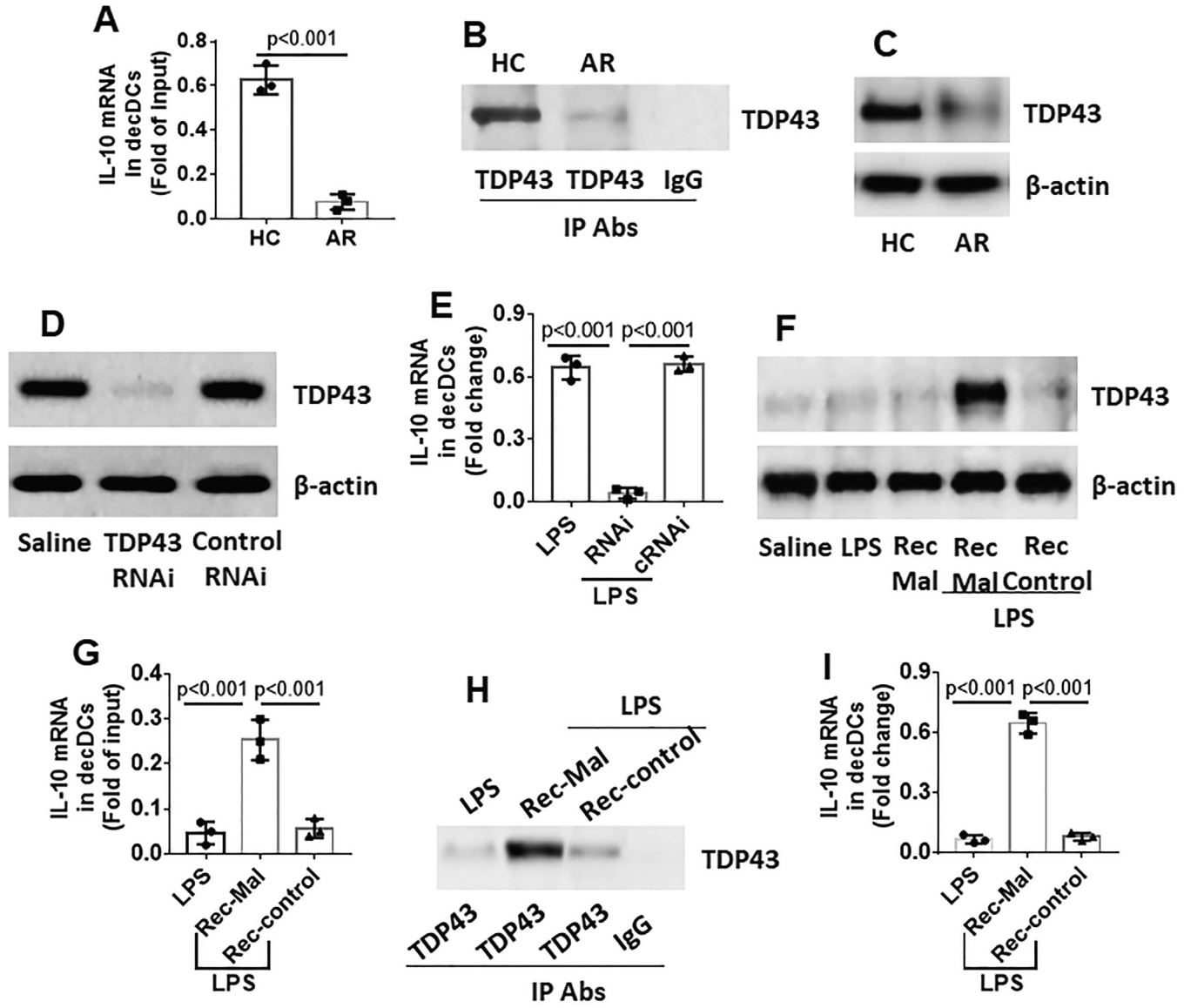

Fig. 5. TDP43 protects IL-10 mRNA from decay in decDCs. A-B, decDCs were isolated from PBMCs collected from blood samples of AR patients (n = 20) and HC subjects (n = 20). Bars indicate IL-10 mRNA and immunoblots indicate TDP43 protein in a complex in decDCs. C, Western blots show TDP43 protein levels in decDCs. D, results of TDP43 RNAi in HC decDCs. E, bars indicate IL-10 mRNA in HC decDCs with or without TDP43-deficiency after exposure to LPS in the culture. F, immunoblots indicate TDP43 levels in AR decDCs after treating with the procedures denoted below the blots. G-H, bars indicate IL-10 mRNA and immunoblots indicate TDP43 protein in a complex in AR decDCs with or without reconstitution of Mal after exposure to LPS in the culture for 48 h. I, bars indicate IL-10 mRNA levels in AR decDCs with or without reconstitution of Mal after exposure to LPS in the culture for 48 h. Data of bars are presented as mean ± SEM. Each dot inside bars present data obtained from an independent experiment. Data of immunoblots are from one experiment representing 3 independent experiments.

10 mRNA was still increased in Mal-deficient decDCs while the protein of Mal was not increased (Fig. 4B-C). On the other hand, reconstitution of Mal in AR decDCs (Fig. 4D) restored the responsiveness to LPS in the expression of IL-10 (Fig. 4E) as well as in the induction of Tr1 cells (Fig. 4F-G). The results demonstrate that Mal plays a critical role in the expression of IL-10 in decDCs.

3.6. TDP43 mediates the effects of Mal on stabilization of IL-10 mRNA in decDCs

Previous reports indicate that TDP43 protects the IL-10 mRNA from decay [23]. We also found a complex of TDP43/IL-10 mRNA in HC decDCs, the amounts of which were significantly less in AR decDCs (Fig. 5A-B). By Western blotting analysis, we observed that the TDP43 protein levels were lower in AR decDCs than HC decDCs (Fig. 5C). The results suggest that TDP43 may play an important role in protecting IL-10 mRNA from decay in decDCs. To test it, TDP43 expression was depleted in HC decDCs by RNAi (Fig. 5D); it resulted in IL-10 mRNA decay (Fig. 5E). Exposure to LPS increased the expression of TDP43 in HC decDCs, which was lower in AR decDCs, but could be up regulated by reconstitution of Mal (Fig. 5F). The levels of TDP43/IL-10 mRNA complex in AR decDCs were also increased by reconstitution of Mal (Fig. 5G-H), followed by stabilization of IL-10 mRNA in AR decDCs (Fig. 5I). The results demonstrate that Mal mediates LPS signal to induce TDP43 expression in decDCs; TDP43 forms a complex with IL-10 mRNA to protect IL-10 mRNA from decay.

3.7. Mal-deficiency in DCs worsens AR response in mice

To further test the role of deficiency of Mal in the pathogenesis of allergic disorders, a mouse strain carrying Mal-knockout decDCs was developed. The number of decDCs in spleen cells was comparable

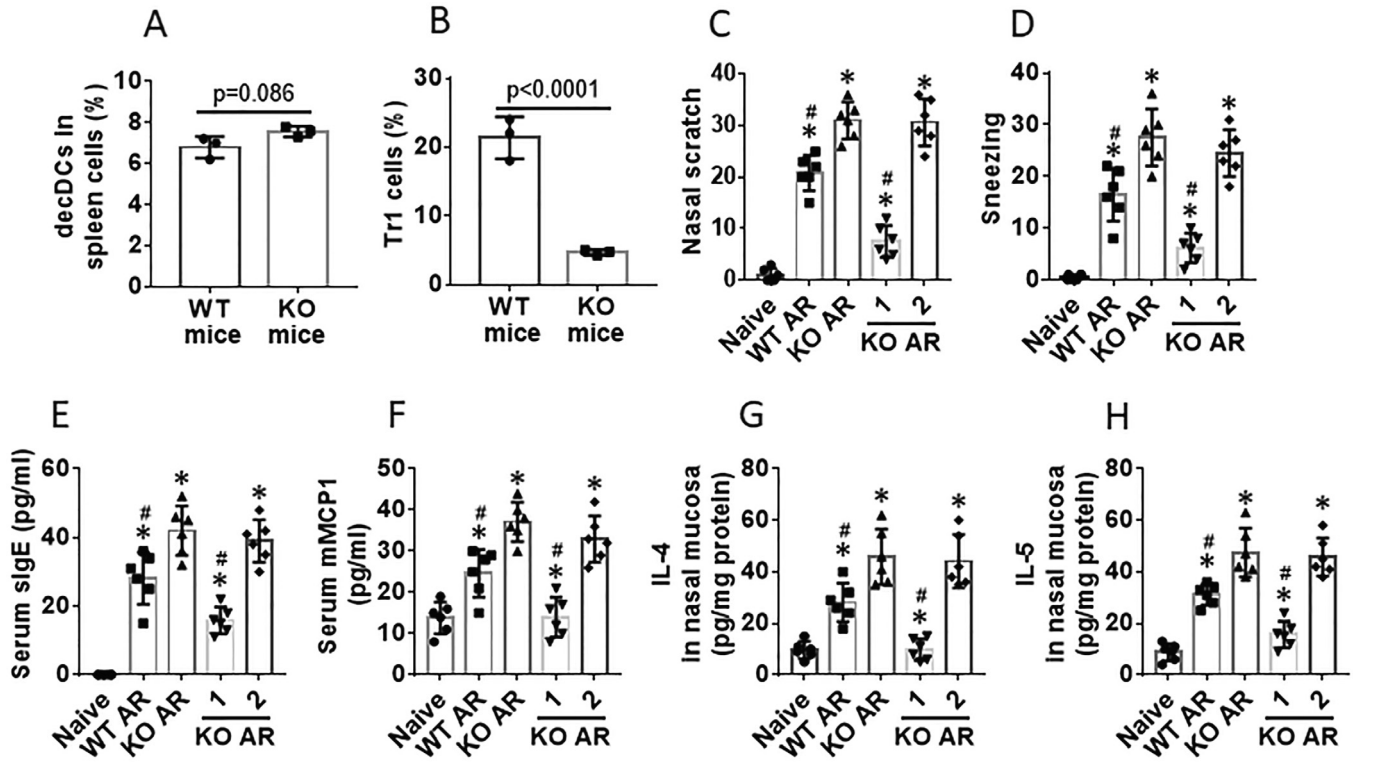

Fig. 6. Mal-knockout decDCs worsen AR response. A, spleen cells were isolated from mice carrying Mal-knockout decDCs (KO) and wild type (WT) mice and analyzed by flow cytometry. Bars show percentage of DEC205$^+$ CD11c$^+$ decDC number in total analyzed spleen cells. B, decDCs and naïve CD4$^+$ T cells were isolated from spleen cells by MACS and cocultured at a ratio of 1:5 for 6 days in the presence of LPS (100 ng/ml). The cells were harvested and analyzed by flow cytometry. Bars show converted Tr1 cells. Dot plots of panel A-B are presented in Figs. S1 and S2) in supplemental materials. C-D, bars indicate records of nasal itch (nasal scratch) and sneezing of mice during 0–30 min after the last antigen challenge. E-F, bars indicate serum levels of specific IgE and mouse mast cell protease-1 (mMCP1). G-H, bars indicate levels of IL-4 and IL-5 in nasal mucosal protein extracts. KO AR: KO mice with AR. WT AR mice: WT mice with AR. 1: KO mice were passively transplanted with Mal-sufficient decDCs. 2: KO mice were passively transplanted with Mal-deficient decDCs. Data of bars are presented as mean ± SEM. Each dot inside bars presents data obtained from an independent experiment.

between Mal-deficient DCs (KO mice) mice and wild type (WT) mice (Fig. 6A; Fig. S2), while the Tr1-generating ability was significantly weaker in decDCs collected from KO mice than that in WT mice (Fig. 6B; Fig. S3). KO mice and WT mice were treated with published procedures [24] to develop AR. WT AR mice showed AR-like response, including nasal itch, sneezing, increases in levels of specific IgE, Th2 cytokines and mouse mast cell protease-1. The AR response was much worse in KO AR mice. Adoptive transplantation with Mal-sufficient decDCs in the presence of TLR agonists suppressed AR response (Fig. 6C-H). The results demonstrate that Mal-deficiency impairs decDCs' immune tolerogenicity.

4. Discussion

While the dysfunction of immune regulation contributing to the pathogenesis of many immune diseases has been recognized, it is urgent to elucidate its causative factors. The present data show that absence or insufficiency of Mal signal can result in immune intolerance by inducing immune regulatory cell dysfunction. The data show that decDCs from HC subjects express IL-10 and can convert naïve CD4$^+$ T cells to Tr1 cells. This fraction of DCs can be regarded as tolerogenic DCs. AR decDCs express much less IL-10 than that of HC decDCs although the number of decDCs is comparable between AR patients and HC subjects. AR decDCs are incompetent to convert naive CD4$^+$ T cells to Tr1 cells. AR decDCs express lower levels of Mal, which is insufficient to induce TDP43 and to prevent IL-10 mRNA from decay. Mice carrying Mal-deficient decDCs show much worse AR response.

Cumulated reports indicate that targeting DEC205 of DCs to deliver specific antigens can induce antigen specific Tregs to contribute to the induction or/and maintenance of immune tolerance [11]. The present data show that the frequency of decDCs is comparable between the AR group and the HC group, suggesting that AR environment may not affect the decDC development in the body. DEC205 on DCs can efficiently take antigens into DCs. After processing, DCs deliver the antigen information to T cells to induce antigen specific effector T cell tolerance [11,25] as well as inducing Foxp3$^+$ Tregs [26], the underlying mechanism is not fully understood yet. The present data add novel information to this area by showing that decDCs can induce Tr1 cells, which suggests that decDC-derived cytokines, it is probably IL-10, induce Tr1 cells.

The present data show that HC decDCs express IL-10 and much less expression of IL-10 in AR decDCs. IL-10 is one of the immune regulatory molecules. IL-10-producing bone marrow derived DCs can convert naïve CD4$^+$ T cells to Tr1 cells [27] while most reports regarding decDC immune tolerogenic property are shown by inducing antigen effector T cell anergy or tolerance [11,25]. Our data indicate that decDCs express IL-10 by which decDCs convert naïve CD4$^+$ T cells to Tr1 cells. Our data demonstrate that AR decDCs are incompetent to induce Tregs as a lower expression of IL-10 is observed in AR decDCs.

The data show that the expression of Mal is lower in AR decDCs. Mal is a common checkpoint at the signal transduction pathway of most TLRs. Although TLR4 signals mainly activate MyD88, instead of Mal, to activate the downstream of the signal transduction pathway, the present data show that the amounts of MyD88 in AR decDCs are not different from HC decDCs. The data show that the expression of Mal in AR decDCs is lower as compared to that in HC decDCs, indicating that the lower expression of Mal may be attributed to the incompetence of inducing Tr1 cells by AR decDCs.

We observed IL-10 mRNA decay in decDCs. RNA decay is a common phenomenon in the cell. It is necessary to stabilize mRNA in the cell until synthesizing enough target proteins. We found less amounts of complex of TDP43/IL-10 mRNA as well as less expression of TDP43 in AR decDCs. TDP43 is a DNA- and RNA-binding protein. Published data indicate that TDP43 protects the IL-10 mRNA from decay [23]. It has recognized that posttranscriptional modifications of mRNAs is a dominant influence in the expression of immune regulatory factors [28]. Our data show that TDP43 is required in posttranscriptional process of IL-10 in decDCs. Although AR decDCs still express IL-10 mRNA upon exposure to LPS, an IL-10 inducer, the IL-10 mRNA decays quickly in decDCs. The Mal signals induce TDP43 expression to prevent the IL-10 mRNA decay in HC decDCs, while after reconstitution of Mal also gains the expression of TDP43 expression and protects the IL-10 mRNA from decay in AR decDCs. The data suggest that activation of MyD88 can induce the transcription of IL-10, while activation of Mal is required in stabilizing the IL-10 mRNA in decDCs through induction of TDP43 expression.

AR is one of the allergic diseases. The prevalence of allergic diseases increased rapidly in the last a few decades [29]. Such a phenomenon is explained by the hygiene hypothesis: because of less exposure to microbial stimuli results in Th2 polarization and prone to suffer from allergic diseases [30]. However, in the recent years, the prevalence of Th1-dominant diseases, such as inflammatory bowel disease, were also increased rapidly [31]. Thus, we may propose that less exposure to microbial stimuli results in the immune dysregulation in the body. The present data show that much less expression of Mal in AR decDCs. Mal is an important component in the signal transduction pathway of TLR4, one of the major sensors to microbial stimuli. Therefore, the less or insufficient expression of Mal is somewhat like less exposure to microbial stimuli. Based on this, we may envision a scenario that less expression of Mal results in less expression of TDP43 in decDCs and IL-10 mRNA decays. The decDCs are thus incompetent to induce or/and maintain Tr1 cells that contributes to immune dysregulation.

In summary, the present data show that AR decDCs express less Mal and are incompetent to induce Tr1 cells. Reconstitution of Mal expression restores the immune tolerogenic properties in AR decDCs. To regulate the expression of Mal in decDCs has a translational potential in the treatment for AR and other allergic diseases.

Author contributions

JBS, GY, YYZ, FM, XQL, LHM, ZQL and WJL performed experiments, analyzed data and reviewed the manuscript. XWZ, DBL and PCY organized and supervised experiments. PCY designed the project and prepared the manuscript.

Declaration of Competing Interest

None.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cellimm.2019.103930.

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